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Effect of Hydrogen Cyanide and Carbonyl Sulphide on the Germination and Plumule Vigour of Wheat

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Abstract: Several factors which may influence the germination of wheat fumigated with hydrogen cyanide or carbonyl sulphide were investigated. Dosages of hydrogen cyanide ranged from 10 mg litre^{-1} for 24-h exposure up to 150 mg litre^{-1} for 96-h exposure. Dosages of carbonyl sulphide ranged from 25 mg litre^{-1} for 24-h exposure up to 500 mg litre^{-1} for 72-h exposure. The experiments were conducted on wheat of 11·4, 13·8 and 15·7% moisture content. The higher levels of these fumigants exceed those needed for control of insects in wheat. Germination was not diminished and may have been slightly enhanced with hydrogen cyanide, but was diminished by high levels of carbonyl sulphide in the drier wheat. The plumule length was reduced following all dosages of hydrogen cyanide, but only after high dosages of carbonyl sulphide, especially on the driest wheat. It is concluded that hydrogen cyanide and carbonyl sulphide could be used to control insects in wheat without affecting seed viability, provided that concentrations are carefully controlled.

Key words: viability, wheat, carbonyl sulphide, hydrogen cyanide.

1 INTRODUCTION

Disinfestation of stored products using fumigants is widely used for the control of insects, rodents and fungi in commodities, buildings and soils. Because fumigation is often the cheapest and most effective process available, it plays a major role world-wide in preserving commodities.

Every fumigant has some constraint on its use; most are highly toxic to mammals and many are flammable. Many fumigants, including acrylonitrile, ethylene dibromide and ethylene oxide, have been withdrawn because of problems with human toxicity.[1] The choice of fumigants for grain is very restricted in commercial practice, with usually only two, methyl bromide and phosphine, commonly considered.[2] Methyl bromide is now under threat of withdrawal because it apparently depletes the Earth's ozone layer. There is therefore a need for new fumigants with potential to replace methyl bromide for fumigation of grain, stored products, timber, and for quarantine treatment.

For the preservation of seed grain, it is essential that the seed is tolerant to the fumigant. Seed viability is also a good indicator of grain quality.[3] In general, fumigants are more likely to harm viability where high dosages and long exposure periods are used[4] or, at least for some fumigants, when the moisture content of the grain is high.[5] Therefore, in this study, the concentration of fumigant, the exposure period and the moisture content of the wheat were varied. Three measurements related to seed quality were investigated. These were seed vigour (germination after four days), ultimate germination (after eight days) and plumule length, which is a measure of the soundness of the initial seedling.

Two fumigants were investigated; hydrogen cyanide (HCN) and carbonyl sulphide (OCS), each a potential alternative to methyl bromide for controlling insects in stored grain. HCN was formerly used as a grain fumigant, but is little used today. On the other hand OCS

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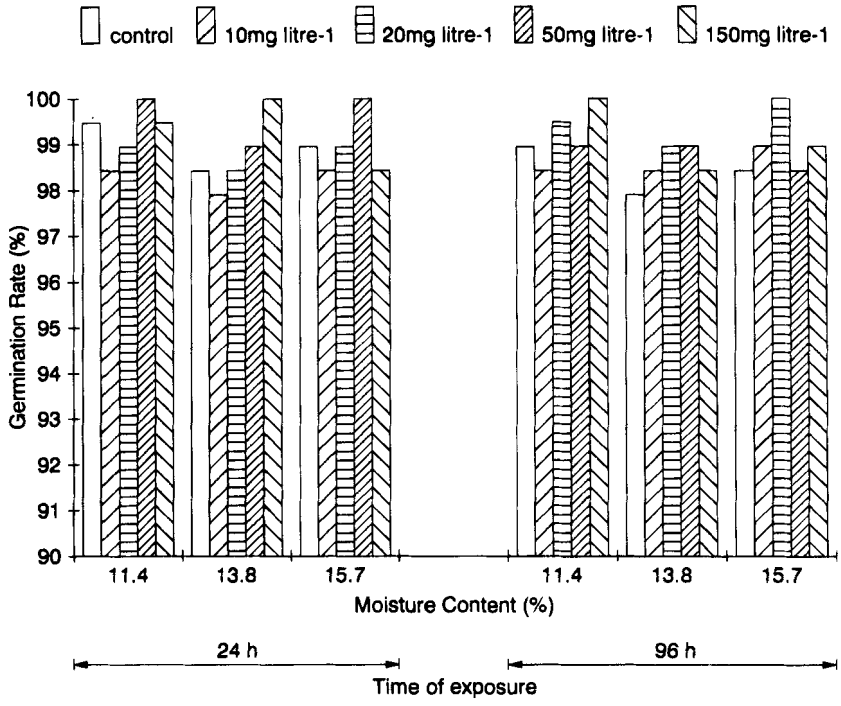

Fig. 1. Percentage germinability of wheat at three moisture contents fumigated with HCN gas at different dosages for 24 and 96 h exposures.

has recently been investigated as a potential new fumigant.[1]

2 MATERIALS AND METHODS

The wheat used was insecticide-free Australian Standard White (ASW, approximately 11·4% moisture content, w/w, wet basis; i.e. m.c.). Portions were conditioned to equilibrium after adding appropriate quantities of water. After one week at 25(±1)°C, samples were found to have moisture contents of 11·4, 13·8 and 15·7%, with corresponding equilibrium relative humidities (e.r.h.) of 47·3, 69·8 and 80·6%. Moisture content (wet basis) was calculated from the loss of mass of ground samples by oven drying at 130°C for 2 h. The e.r.h. was calculated from a measured equilibrium dew point observed on a cooled mirror dew point meter (MBW Elektronic AG, model DP3-D) placed in a closed loop with a 1-kg wheat sample.

2.1 Fumigation apparatus and treatment

Conditioned wheat samples (40 g) were exposed at 25(±1)°C to HCN gas generated *in situ* in 120-ml vials (filling ratio of approximately 0·50) using potassium cyanide and hydrochloric acid (1 M). Calculated amounts of potassium cyanide were weighed into small Petri dishes, covered with filter paper and transferred to vials. Each was sealed with a Mininert cap, and the hydrochloric acid was introduced by syringe. Four levels of HCN and a control were used (0, 10, 20, 50 and 150 mg litre^{-1}) for each of three wheat samples of different moisture content, and for two different periods of exposure (24 and 96 h) at 25(±1)°C. Before germination, wheat was transferred to Petri dishes and aired for 24 h.

Conditioned wheat samples (40 g) were exposed at 25(±1)°C to OCS in 120-ml vials capped with a Mininert injection system. OCS (90% purity, v/v), generated through the reaction of potassium thiocyanate and 9 M sulphuric acid,[6] was introduced by a gas syringe. Different levels of OCS and a control (0, 25, 50, 100, 250 and 500 mg litre^{-1} corrected level) were applied to each of three wheat samples of different moisture content, and for three different periods of exposure (24, 48 and 96 h).

2.2 Germination tests

Germination tests were carried out according to the principles stated in International Seed Testing Association Methods,[7] adapted by Ghaly *et al.*[8] Fifty seeds were saturated with approximately 40 ml of distilled water and wrapped in two rolled crepe filter papers (500 × 330 mm each). The seeds were arranged 3 cm apart on the top half of the sheet (i.e. 250 × 330 mm), using a seed counting board, and the lot covered by folding the lower half over them. Each doubled sheet was saturated with water and loosely rolled from one side to the other, perpendicular to the base. It was then

held together with a rubber band and put in an upright position in the germination cabinet, at 25°C. The number of germinated seeds was counted after four days (vigour test) and after eight days (total germination test) and the average plumule length was determined at eight days. Each experiment was also replicated four times. The data were analysed statistically for standard error and variance.[9]

3 RESULTS AND DISCUSSION

3.1 Hydrogen cyanide fumigation

The germination rate of wheat after exposure to HCN is shown in Fig. 1. The standard error from four replicates of 50 seeds each was less than 1% in all cases. Doses ranging from 10 to 150 mg litre^{-1} for 96 h did not diminish the germination potential and may have slightly enhanced it in comparison with unfumigated seed. Results from the vigour test (four-day count) were unchanged at eight days. Thus HCN had no deleterious effect on either the speed or the extent of germination, under any of the conditions tested.

The standard error in plumule length was less than 4% of the mean value in all cases. However, HCN had an effect on plumule length at exposure periods of 24 and 96 h (Fig. 2), and in all cases, higher doses of HCN reduced plumule length.

A number of publications have examined the effect of HCN on seed germination, partly from the viewpoint of its natural role in germination. Roberts[10,11] reported that rice and barley germination was stimulated by pretreatment with KCN solution but the extent was not quantified. *Sesbania* and *Coriander* seed fumigated with HCN gas at 48 mg litre^{-1} for 2 h at 27°C under a reduced atmosphere (200 mm of mercury, conditions which would be effective in killing insects present in the seed) showed no significant decrease in germination rate.[12] A large proportion of endogenous HCN is normally liberated from cyanogenic glycosides by β-glucosidase. Esashi *et al.*[13] reported that HCN was effective in stimulating the germination of upper cocklebur seeds. This is to be expected; though the cytochrome pathway is inhibited by the action of HCN, unlike carbon monoxide, alternative respiration pathways are adequate for germination.[14] In addition to this mechanism, cyanide groups may bind to other (unknown) compounds necessary for germination. Despite the 'natural' role of HCN, and its lack of inhibition of germination at higher concentrations, increasing doses significantly reduced the plumule vigour of fumigated seeds (Fig. 2).

Thus, absence of a decrease in germination rate, or even germination vigour, after fumigation is insufficient to prove a lack of effect on seed quality. It is necessary

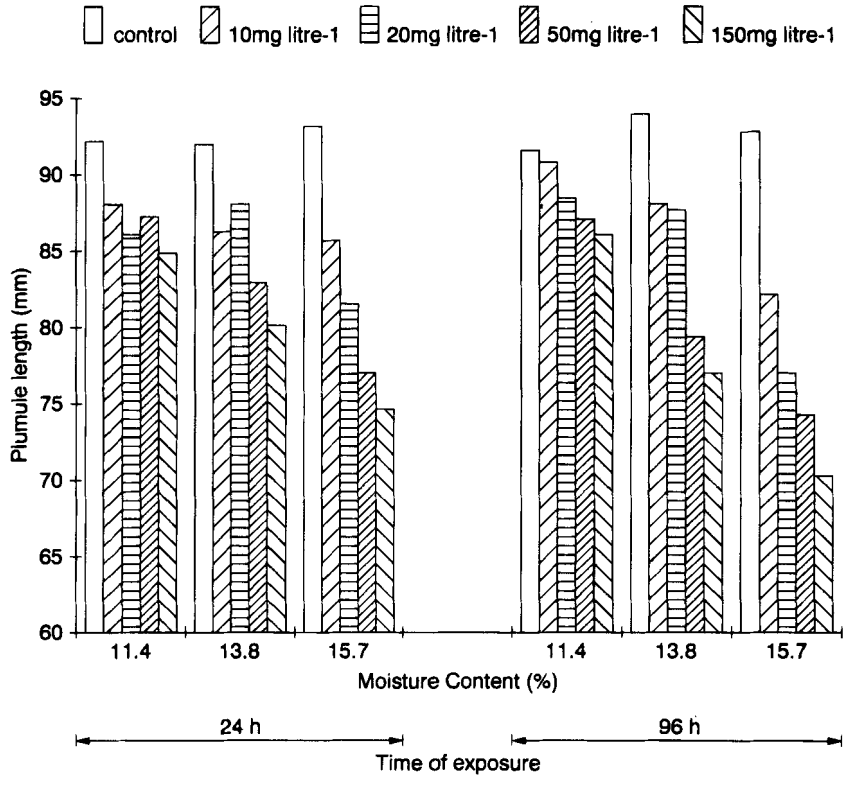

Fig. 2. Plumule length of wheat at three moisture contents fumigated with HCN gas at different dosages for 24 and 96 h exposures.

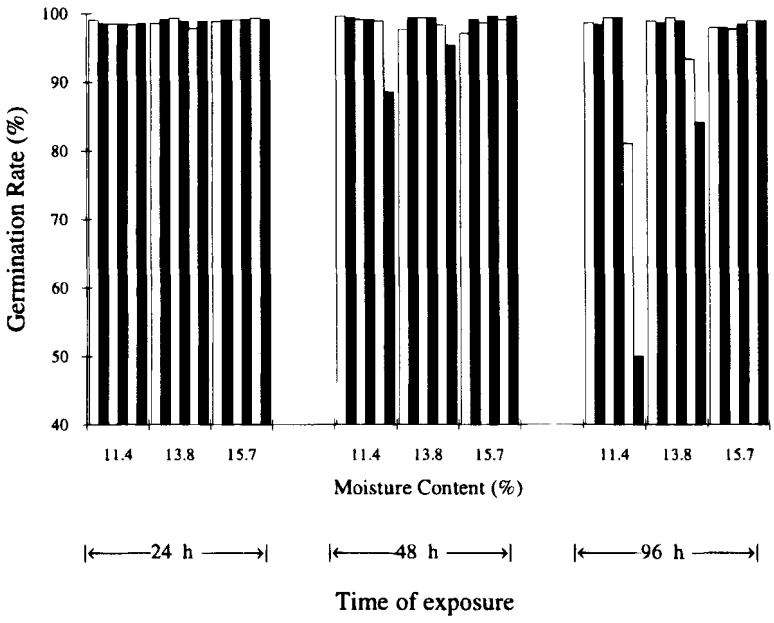

Fig. 3. Percentage germinability of wheat at three moisture contents fumigated with OCS at different dosages for three exposure periods. For each moisture content, the bars represent, in order from left to right: (□) control, (■) 25 mg litre^{-1}, (□) 50 mg litre^{-1}, (■) 100 mg litre^{-1}, (□) 250 mg litre^{-1}, (■) 500 mg litre^{-1}.

to determine the growth of the plumule. However, it is not yet known what relationship exists between the plumule length after eight days and the final yield of grain from the plant.

3.2 Carbonyl sulphide fumigation

At 24 h exposure, the germination rate of wheat was unaffected at all moisture contents and by fumigant dosages tested (Fig. 3). The standard error from four replicates of 50 seeds each was less than 1%. At 48 and 96 h exposure, the germination rate dropped at the higher levels of OCS (250 and 500 mg litre^{-1}) when the moisture content of the wheat was lower (11·4 or 13·8%, Fig. 3) but not when it was higher (15·7%). This result is in contrast to the effect of carbon disulphide on germination, where germination rate decreases with increasing moisture content.[6] One possible explanation for these results is the suppression of fungi by OCS, especially in high moisture wheat. OCS suppressed fungal growth relative to the control in a manner which increased with, (a) dose, and surprisingly, (b) moisture content conditions (Table 1). No fungal growth was detected at levels of OCS of 250 mg litre^{-1} or above in wheat of 15·7% m.c., whereas over 40% of unfumigated seeds showed black mould marks after germination (Table 1). Whether OCS, or a product formed from its degradation, is responsible as the inhibitor was not determined.

TABLE 1
Level of Mould Growth on Germinated Wheat Exposed to Different Levels of OCS in Air[a]

	Moisture content (%)		
Dosage (mg litre^{-1})	*11·7*	*13·8*	*15·7*
0 (Control)	††	††	†††
25	††	††	††
50	††	††	†
100	††	†	†
250	†	†	×
500	†	×	×

[a] †††—more than 40% germinated wheat seeds with black mould marks.
††—40–20% germinated wheat seeds with black mould marks.
†—less than 20% germinated wheat seeds with black mould marks.
×—no mould marks.

The standard error in plumule length was less than 4·5% of the mean value in all cases. The plumule length of wheat followed a trend closely analogous to the germination rate (Fig. 4). That is, no significant effect on plumule length resulted from a 24-h exposure at the lower levels of either OCS or moisture content. Exposure of the drier wheat to the higher concentrations for longer periods reduced plumule length.

Both HCN and OCS can be used without affecting germination, at levels needed to control insects. Unlike HCN, OCS can be used at efficacious levels without decreasing plumule length. Attention to details of

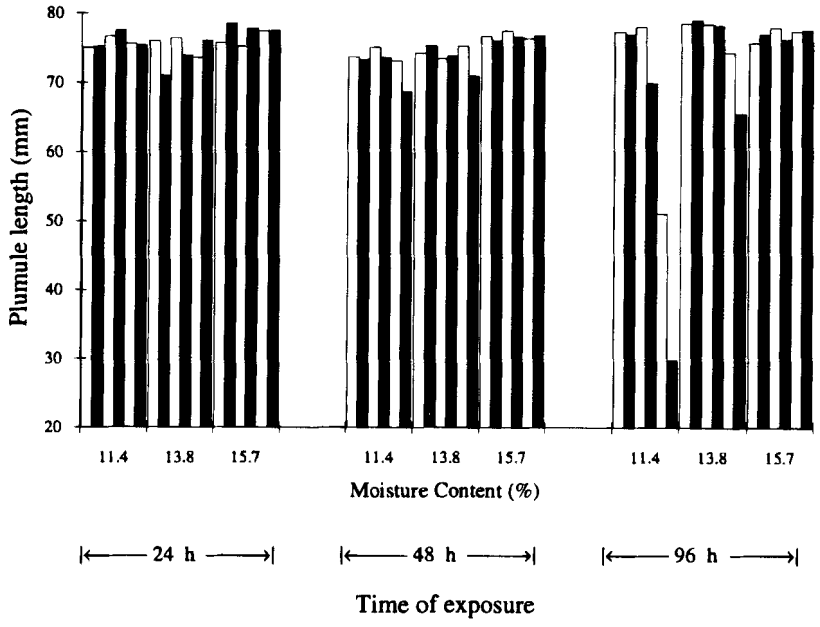

Fig. 4. Plumule length of wheat at three moisture contents fumigated with OCS at different dosage for 24, 48 and 96 h exposure. For each moisture content, the bars represent, in order from left to right: (□) control, (■) 25 mg litre^{-1}, (□) 50 mg litre^{-1}, (■) 100 mg litre^{-1}, (□) 250 mg litre^{-1}, (■) 500 mg litre^{-1}.

dosing is required. The mould-inhibitory action of OCS merits further investigation.

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